

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY



(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 23 JUN 2005

WIPO

PCT

Applicant's or agent's file reference GIP25PT04		<b>FOR FURTHER ACTION</b>		See Form PCT/PEA/416
International application No. PCT/EP2004/051491		International filing date (day/month/year) 14.07.2004		Priority date (day/month/year) 17.07.2003
International Patent Classification (IPC) or national classification and IPC C12P19/04, C08B11/20				
Applicant LAMBERTI SPA et al.				
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau a total of 2 sheets, as follows:</p> <p><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (Indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>				
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input checked="" type="checkbox"/> Box No. VIII Certain observations on the international application</p>				
Date of submission of the demand  18.03.2005		Date of completion of this report  21.06.2005		
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized Officer  Fausti, S Telephone No. +49 89 2399-7389 		

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/EP2004/051491

---

**Box No. I Basis of the report**

---

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language, which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
  - ☐ publication of the international application (under Rule 12.4)
  - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements\*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

**Description, Pages**

1-7 as originally filed

**Claims, Numbers**

1-7 received on 18.03.2005 with letter of 28.02.2005

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to sequence listing (*specify*):

\* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/EP2004/051491

---

**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

---

**1. Statement**

Novelty (N)	Yes: Claims	1-7
	No: Claims	-
Inventive step (IS)	Yes: Claims	1-7
	No: Claims	-
Industrial applicability (IA)	Yes: Claims	1-7
	No: Claims	-

**2. Citations and explanations (Rule 70.7):**

**see separate sheet**

---

**Box No. VIII Certain observations on the international application**

---

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability;  
citations and explanations supporting such statement**

**1. DOCUMENTS and ABBREVIATIONS.**

**CMC:** Carboxymethyl cellulose  
**Dex:** Dextran  
**PEG:** Polyethylen glycol  
**DS:** Degree of Substitution

- 1.1** D1 and D2 disclose methods for reducing the viscosity of CMC by means of cellulase-mediated hydrolysis (see abstracts). In particular aqueous CMC solutions of low-viscosity CMC are prepared according to these methods (see: D1, examples 3-4; D2, example 1).
- 1.3** D3 discloses a method for reducing the viscosity of CMC by means of peroxide oxidation (see abstract). In a specific embodiment, a CMC composition containing 31.1% cellulose of low viscosity is produced (see example 1).
- 1.4** D4 suggests that CMC having an etherification degree of at least 1.4 is not enzymatically hydrolysed and maintains therefore the initial viscosity (see the Derwent abstract).
- 1.5** D5 discloses a method for reducing the viscosity of cellulose ether derivatives by irradiating high molecular weight cellulose derivatives in the presence of a base (see the abstract, the third paragraph on page 3 and examples 1-6). This method leads to compositions of low-viscosity cellulose which are stable over time (see paragraph joining pages 2 and 3).
- D6** D6 discloses methods for enzymatic cellulose hydrolysis in an aqueous Dex/PEG biphasic system for enzyme recycling (see abstract).
- D7** D7 teaches that cellulase activity in CMC hydrolysis is not influenced by the presence

of an alcohol component in the fermentation mixture (see abstract).

**2. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).**

- 2.1** Claims 1-7 relate to low-viscosity CMC solutions and methods for their preparation, which can be made or applied in industrial productions and are therefore considered industrially applicable according to article 33(4) PCT.

**3. NOVELTY (Art. 33(2) PCT) and INVENTIVE STEP (Art. 33(3) PCT).**

- 3.1** The subject-matter of claims 1-7 is novel over the available prior art in view of the alcoholic solvent component used in the dissolution and hydrolysis steps. The prior art does not disclose any method of enzymatic CMC depolymerization for the preparation of low-viscosity CMC solutions, wherein CMC of medium-viscosity is dispersed in a hydro-alcoholic mixture and subjected to cellulase activity (see points 1.1, 1.3, 1.5-1.7 above).
- 3.2** D1 and D2 can independently be considered to represent the relevant state of the art because they disclose methods of enzymatic CMC hydrolysis for reducing the viscosity of aqueous cellulose solutions, from which the subject-matter of claims 1-7 differs in the presence of an alcohol component in the solvent mixture.
- 3.2<sup>a</sup>** The problem to be solved can therefore be regarded as the provision of an alternative (improved) method of enzymatic CMC hydrolysis for reducing the viscosity of CMC solutions.
- 3.2<sup>b</sup>** The claimed subject-matter is considered to involve an inventive step over D1 and/or D2 because the distinguishing feature of the alcohol component in the solvent mixture has not been suggested for processes of enzymatic cellulose depolymerization. D7, which teaches that cellulase enzymes can work in alcoholic media, relates to the use of cellulose as a source of glucose and reducing sugars in the fermentative production of ethanol (see point 1.7 above). Hence, D7 does not suggest the use of an hydro-alcoholic solvent system in cellulose hydrolysis for the purpose of reducing the rheological properties of this carbohydrate polymer.

Moreover, the presence of the alcohol component in the solvent mixture for CMC dissolution and hydrolysis leads to an unexpected improvement over the prior art methods (see D1 and D2), namely a better storage stability of the CMC solutions produced (see the last sentences of examples 1-3).

- 3.2° In addition, the degree of substitution of the CMC substrate, which is used as starting material in the claimed processes, is specifically comprised between 0.5 and 1.0. This relevant feature excludes from the claimed scope the CMC substrates with high degree of etherification, which do not appear to solve the problem posed. For example, the prior art discloses that no enzymatic hydrolysis of CMC substrates with high degree of etherification takes place, and therefore the CMC viscosity is not reduced (see point 1.4 above).

**Re Item VIII**

**Certain observations on the international application**

**C. CLARITY (Art. 6 PCT).**

- C.1 The statement in the description on page 21 (see lines 19-22) is inconsistent with the claims in that it presents some of the technical features of the claimed processes as preferred, rather than as essential (see "normally" for the degree of substitution and "preferably" for the viscosity). According to this passage in the description, the subject-matter for which protection is sought might be different to that defined by the claims, thereby resulting in lack of clarity. In the present case, the lack of clarity is relevant because novelty and inventive step are addressed to these technical features (see above).

**Claims**

1. Process for the preparation of an aqueous solution of carboxymethylcellulose containing from 20 to 40 wt% of carboxymethylcellulose, having Brookfield viscosity at 20 °C and 20 rpm from 2000 and 5000 mPa\*s, characterised by the fact that it comprises the following steps:
  - a. from 20 to 30 pbw (parts by weight) of carboxymethylcellulose having a degree of substitution comprised between 0.5 and 1.0 and whose aqueous solutions at 4 wt% have Brookfield viscosity from 20 to 1000 mPa\*s, at 20°C and 20 rpm are dispersed in 100 pbw of a mixture of water and alcohol containing from 30 to 60 wt% of alcohol;
  - b. the obtained dispersion is heated at a temperature of 35-55 °C, 0.5 to 10 pbw (each 100 pbw of carboxymethylcellulose) of a cellulase preparation are added, and the mixture is stirred at this temperature for 60-200 minutes;
  - c. the alcohol is removed by distillation;
  - d. the cellulase preparation is deactivated by alkalinising and heating at 60-70°C for 20-120 minutes;
  - e. after cooling at 40-55 °C, from 1 to 5 pbw (each 100 pbw of carboxymethylcellulose) of a 30-35 wt% aqueous solution of hydrogen peroxide are added, the mixture is stirred at 55-70°C for 15-45 minutes, optionally adjusting the carboxymethylcellulose concentration by adding water.
2. Process for the preparation of an aqueous solution of carboxymethylcellulose according to claim 1., wherein the alcohol is ethanol or isopropanol.
3. Process for the preparation of an aqueous solution of carboxymethylcellulose according to claim 2., wherein the carboxymethylcellulose which is dispersed in step a. has a degree of substitution comprised between 0.6 and 0.8.
4. Process for the preparation of an aqueous solution of carboxymethylcellulose according to claim 3., wherein the carboxymethylcellulose which is dispersed in step a. has a Brookfield viscosity at 4 wt% comprised between 20 and 500 mPa\*s, at 20°C and 20 rpm.

5. Process for the preparation of an aqueous solution of carboxymethylcellulose according to any of the preceding claims, wherein the cellulase preparation of step b. is preparation of cellulase containing natural cellulase complexes having endoglucanase activity (EG-I, EG-II, EGIII), exoglucanase activity (CBH-I and CBH-II) and  $\beta$ -glucosidase activity.
6. Process for the preparation of an aqueous solution of carboxymethylcellulose according to any of the claims from 1. to 4., wherein the cellulase preparation of step b. is selected among a preparation of cellulase without CBH-I but EG-I and EG-II enriched, a preparation of cellulase having a single EG-III activity expressed by a cloned gene, and mixture thereof.
7. Process for the preparation of an aqueous solution of carboxymethylcellulose according to any of the preceding claims, wherein the mixture of water and alcohol contains from 40 to 50 wt%, of alcohol.